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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 804

Contribution from the Bureau of Entomology
L. O. HOWARD, Chief

Washington, D. C. PROFESSIONAL PAPER March 16, 1920

A STUDY OF THE BEHAVIOR OF BEES IN COLONIES AFFECTED BY EUROPEAN FOULBROOD[1]

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INTRODUCTION

The brood diseases of bees cause annually large losses of bees and consequently of the honey crop. The predominant attitude among beekeepers has long been how best to eradicate an invading bee disease after the attack has been made. They depend upon this procedure, because little is known with any degree of certainty concerning the natural conditions which might prevent or control the onslaught of the disease. As a result of this attitude, much more importance has been placed on the significance of apiary inspection and police-power laws and of purely remedial treatment, the reasons for which in many cases are imperfectly understood. But the old adage "an ounce of prevention is worth a pound of cure" has yet to be refuted, particularly with regard to beekeeping. In the realm of human

[1] A series of investigations was started in the spring and summer of 1918 by the Office of Bee-Culture Investigations, Bureau of Entomology, for the purpose of making an intensive study of European foulbrood of bees, primarily from the standpoint of insect behavior in relation to the disease, correlated with the facts and practical observations already known to the beekeeper. This paper, which was submitted for publication January 13, 1919, is a preliminary report on the beginning of the investigation.

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medicine, for the last two decades at least, this precept has been gaining strength so that to-day preventive medicine stands on a par with, if not above, most of the other branches of medicine. Why is it not logical to apply this principle to the control of bee diseases?

Ever since European foulbrood of bees was first recognized (in 1894), in New York State, as a distinct brood disease, there have been much controversy and speculation concerning the etiology of the disease, the means of transmission, the method of spread, and, resulting therefrom, the question of control. From the laboratory standpoint, the etiology of the disease has been worked out quite definitely bacteriologically (12)[1]. But as yet *Bacillus pluton*, the accepted cause of European foulbrood, never has been grown in pure culture on artificial media, although it has been definitely identified as the cause of the disease. This precludes any further advance along this line of attack for the time being.

From the side of practical experience, there have been recorded large numbers of observations, many of them of a similar nature. These observations have led to many accepted practices, as, for instance, the use of Italian bees and strong colonies in combating the disease. Although the weight of numbers tends to give substantiation to observations, the scientific explanation of how these things are true never has been studied carefully and coordinated with the practical side into an epidemiological study of the colony under disease conditions in European foulbrood.

The history of bee diseases has developed mainly along two lines. The scientific side has been concerned principally with determining the causes of the various diseases microbiologically, the method of diagnosis, and conclusively differentiating them. These facts have been described sufficiently in various bulletins of the Bureau of Entomology and will not be discussed here. From the practical side, countless observations have been recorded, largely in the bee journals, in which various manifestations of the disease and experiences with methods of treatment have been discussed. But in all this literature, particularly with regard to European foulbrood, there are few observations on the disease and on the behavior of the bees in relation to it beyond simple description of symptoms.

Early in the experience with European foulbrood it was learned by careful observers that strong colonies are essential in successfully combating the disease. Later the value of Italian bees was discovered. West (11), a New York State apiary inspector, in giving what is one of the best early descriptions of European foulbrood, makes some pertinent observations on the disease. He states that when diseased brood is placed above a strong, healthy colony, with a queen excluder between, so that any healthy brood may emerge,

[1] Reference is made by number in parenthesis to "Literature cited," p. 28.

the diseased larvæ are cleaned out as this is taking place. The union with a healthy colony and the strength gained by the emergence of so many young bees gives the colony the stimulus to eliminate the disease. He notes, as have many other beekeepers since, that in August, when the buckwheat honey flow begins, the stronger of the diseased colonies are stimulated to clean up.

Alexander (1) published a method of treatment for European foulbrood, the principle of which, after many varying failures and successes, is now the basis for the present method of treatment most used; that is, requeening with Italian stock. Alexander mentions the need of three factors: First, the necessity of requeening with young yellow Italians, as hybrids of Italian and black bees are prone to contract the disease in the first place and also are more likely to succumb to it; second, particularly emphasized, a period (at least 27 days, according to Alexander) of queenlessness in which to allow the bees properly to clean up the cells and polish them, preparatory for eggs of a new queen; third, a factor which is mentioned only casually but which is equally important with the other two, the direction to unite and strengthen diseased colonies before treating. So little emphasis was placed on this that the majority of beekeepers overlooked it in using Alexander's treatment and therefore condemned the treatment as unsuccessful except in rare cases.

In an editorial (8) in the same issue of the journal in which Mr. Alexander was writing, the question was raised as to why the period of broodlessness caused by winter, which is much longer than 27 days, does not always prevent a recurrence of the disease. Mr. Alexander answered this question by explaining that when the queen stops laying in the fall, the bees do not polish up the cells as they do earlier in the season, and that some of the dried-down material may remain until the next spring. The opinion also is given in this editorial that Italians are more able to resist the disease than hybrids because they do more thorough work in house cleaning and are less inclined to rob.

Phillips (6) makes the statement that "European foulbrood is more destructive during the spring and early summer than at other times, often entirely disappearing during the late summer and early autumn, or during a heavy honey flow," but gives no indication as to how this takes place. The same year Miller (2) published his theory of the relation of the nurse bees to the spread of European foulbrood. He believes that the nurse bees suck up the juices of a freshly diseased larva which has not become offensive, and then transmit the disease when feeding the healthy larvæ. On this supposition he believes that if egg laying ceases for 5 or 6 days ("the period the larvæ remain unsealed in their cells") there will no longer be larvæ in the proper condition for nurse bees to feed upon,

nor healthy unsealed larvæ to receive the infection, and the disease will thereby come to an end.

Dr. Miller has been using a 10-day period of queenlessness in his treatment of European foulbrood since his accidental discovery that 10 days were sufficient, but in a later article (3) in enlarging upon his nurse-bee theory he assumes that the larva is fed during a period of 5 days but is not effective as a carrier of infection during the whole time as probably no larvæ are torn open until they are 2 or 3 days old, thus making it possible to shorten the queenless period even more. He admits that not all the dead, partially dried larvæ will be cleaned out, but believes that it is only the fresh yellow ones which are infectious. He also states that nurse bees are not inclined to travel far on the combs, a fact which may explain why the disease may be found confined to one comb for several days before spreading farther. Dr. Miller seems to have overlooked several important factors which will be discussed later.

Quite an extensive piece of investigation was carried on during the summers of 1915 and 1916 by the author at the Massachusetts Agricultural Experiment Station upon the effect of requeening diseased colonies with various strains of Italian bees. At that time the importance of strong colonies with the requeening had not been emphasized so strongly and less attention was paid to that factor. The records show, however, that in a total of 50 colonies observed, covering two seasons, of 10 strong colonies only 2 showed recurrence, while 1 was doubtful; of 20 medium-strength colonies, 10 showed recurrence with 2 doubtful; of 14 weak colonies, 8 showed recurrence. In all these cases the new queen was not introduced until the colony was nearly or entirely clean. In the case of several of the weaker colonies it was necessary to strengthen them before requeening was possible, in order to save the colony. One or two of these, which were united and requeened with Italian stock, were the best colonies the next spring.

Adding some strength to at least part of Miller's theory is a statement in a letter by G. C. Matthews, formerly of this bureau, who wrote in February, 1918, concerning his observations in California in 1914. He found that where the hives stood in rows of pairs the disease continued to spread down each row to corresponding members of each pair. This ceased when he rearranged his apiary so that the rows of hives were at least 10 feet apart, and alternate pairs of hives were turned at right angles. No pair was allowed to remain close to another facing the same way. This prevented the drifting of nurse bees, which he believes to be the method of spreading the disease. Furthermore, he found by introducing one Italian queen into the middle colony of an isolated row of hybrid bees that there was considerable drifting of nurse bees. Seven days after the brood

from the Italian queen began to emerge, yellow bees were found on either side in several of the hybrid colonies. Speaking of uniting weak diseased colonies and requeening, Matthews writes:

> After two or three were put together, each stack of brood was given an Italian cell. When young queens commenced to lay there was still disease in many of those hives, but as the queens increased in laying the bees cleaned out an ever-increasing sphere of comb for a brood nest until they had the hives free of disease. But in no case, however long a hive might be queenless, did I see the disease cleaned out before a virgin appeared in the hive. In other words, a virgin had to be present before the bees would commence their job of cleaning up. Therefore, I see little to commend the practice of keeping diseased colonies queenless 21 days.

A new bulletin by Phillips (7) has been issued recently by the Department of Agriculture. The fundamental idea emphasized is that "in keeping European foulbrood under control it is far more important to prevent the disease from getting a foothold in a colony than it is to eradicate the disease afterward." This bulletin, aside from discussing symptoms and methods of treatment, states concisely for the first time the facts observed in apiary practice on which successful treatment is based, and without an understanding of which it is difficult for a beekeeper to use preventive measures with any success.

The analysis of these factors of response in behavior to treatment, as stated by Phillips, has been used to some extent as a foundation for the present work on the behavior of the colony in relation to disease, in an endeavor to substantiate, with data obtained under controlled conditions, these facts that are constantly observed in apiary practice and, if possible, to eliminate confusion in methods of treatment.

PROCEDURE

Shortly after the middle of May, 1918, experiments were started in Ithaca, N. Y., at the Cornell Agricultural College. Through the kindness of Prof. J. G. Needham, head of the department of entomology, and others associated with him, the use of a small, isolated yard of bees and also of laboratory facilities was offered for the purpose of carrying on these investigations. This small apiary had been used previously in fruit-pollination studies and had no record of disease. The yard was admirably located in a naturally well-protected hollow beyond the college fruit orchards, about a mile and a half from the main college apiary or other apiaries, with high ground and woods intervening. The author and the Office of Bee-Culture Investigations are under deep obligations to the Cornell authorities for the assistance so cordially extended.

Being in the buckwheat district, the general locality was well adapted to the work because of the desire for as late a main honey

flow as possible in order not to have the influence of a heavy honey flow until other factors had been studied. At Ithaca the main honey flow is generally from buckwheat, coming from the 1st to the middle of August. Rather unfortunately for the best results from the experiments, however, the summer of 1918 was unusual in this section, for the abnormally heavy honey flow from clover necessitated finishing the work earlier than had been planned, owing to the great difficulty of artificially infecting colonies during the heavy honey flow.

There were seven colonies in the original experimental apiary. At first it was intended to work on a larger scale, but the trend of the observations soon led to the plan of working in more detail and on a smaller scale. These colonies were moved some distance apart to prevent drifting and robbing. Some were divided and some were strengthened in an effort to make a series of experiments on colonies of different strengths. The colonies were designated by letter and the combs of each colony by number. From time to time some of these colonies were artificially infected with diseased European foulbrood larvæ from samples sent to the laboratory for diagnosis. Similar colonies were held intact and uninfected for controls. The infection was made by feeding diseased larvæ macerated in sugar solution (about 50 per cent). For the preliminary experiments 10 larvæ were fed in about 250 c. c. of sirup. Later, after the heavy honey flow had begun, it was necessary greatly to increase this dose in order to start the infection. The infected sirup was fed to the bees in sterilized glass petri dishes, placed on top of the frames and protected by an empty comb-honey super placed on the regular hive body with the cover on top.

At the time of inoculation, the condition of each colony was noted as to age, race, condition and appearance of the queen, proportion of nurse bees to old field bees, the number of frames of brood with the amount in each, its age, sealed or unsealed; in other words, the condition of the colony with regard to factors known to be significant in resisting disease. In two colonies the infected sirup was slightly colored with harmless eosin dye to determine where the fresh sirup was placed and its ultimate disposition. At first daily observations were made to determine the earliest appearance of disease, the period of incubation, the symptoms exhibited, and the rate of increase.

By holding up each comb in bright sunlight so that the light shone directly on the larvæ, it was easy to detect the first symptoms of the disease. All the healthy larvæ had the characteristic firm, well-rounded, pearly-white, glistening appearance. The first effect of the disease, besides an abnormal uneasy movement, was a loss of the

glistening character and a slight tinge of grayish or creamy discoloration which would not be noticed except in direct sunlight. These larvæ showed only *Bacillus pluton* present when examined microscopically, as will be mentioned later. Soon after these first symptoms, however, the more noticeable symptoms appeared, such as a larva with its back out, the increase of the light grayish yellow color, and, later, the moist, melting appearance.

A statistical record was kept of the number of larvæ showing new disease at each observation, the number previously diseased that had been cleaned out in the interval since the previous observation, and those remaining over in the cells uncleaned for more than one period between observations. At various times observations were made of the behavior and types of bees engaged in cleaning up and the fate of the material removed. Great care was necessary in these observations to disturb the colony as little as possible. On good days it was sometimes possible to remove a comb carefully from the hive and to watch the bees continuing at their work, and even to watch the queen laying eggs. An eight-frame observation hive containing a strong healthy colony was given a diseased comb from time to time and the bees were observed as they worked on it.

One of the difficulties of the work was to find a satisfactory method of recording the desired data for each comb. At first the diseased cells were marked on the comb by a circle of red celloidin around the entrance of the cell. Although this dried rapidly, it proved unsatisfactory, as the bees, in their attempt to remove the foreign material, seemed to remove both diseased and healthy larvæ indiscriminately. Next small pins were used, inserted in the cell above the one showing disease. In this case the bees tore down the surrounding cells and completely removed the pins, many of which were found on the bottom board. Finally a method of plotting the diseased cells in a comb was adopted. An empty frame was laid off in inch squares by means of heavy black thread. This, used as a templet superimposed on a comb, aided in the location of the diseased and cleaned out cells, so that they could be recorded on a correspondingly ruled card (fig. 1). Placing this over the comb, it was easy to locate exactly each cell and to determine how long the diseased material remained, thus aiding in following the course of the disease throughout its various stages. The only difficulty with this method was the tediousness of the observations. Therefore, after the disease had become definitely established, daily observations of each colony were considered unnecessary. Longer periods showed just as well what was happening in the colony. Also, after the disease had developed enough so that it could be definitely predicted whether the colony would recover or gradually be exterminated, observations of behavior under treatment were

started, the method and degree of house cleaning being watched after the colony had been dequeened, strengthened, and requeened with good Italian stock. Note was also made of any recurrence of disease and

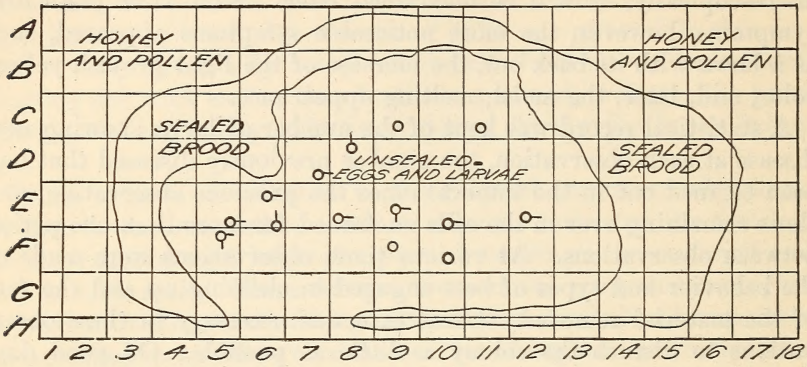

FIG. 1.—Method of plotting the location and history of diseased bee larvæ in the combs. ○ Freshly diseased larvæ. ○- Cells that have been cleaned out. ♀-Cells that have been cleaned out and filled with nectar. ♂ Larvæ remaining in the cells more than one observation period. The area of sealed brood was the amount present at the time of infection of the colony.

apparent reason therefor. In other words, a complete study was made of the cycle of the disease and of the activities of the bees during its course.

OBSERVATIONS

COLONY G

Race.—Hybrid.

Queen.—1917, dark and poor.

Bees.—Workers and drones very dark, almost black, very excitable.

Condition of colony at time of infection.—Brood in four frames, a little less than half sealed, besides two frames of eggs. Bees covering about eight frames, medium strength. Slightly more field bees than nurse bees, because of having divided this colony, old bees returning from the division.

Date of first infection.—May 28, 1918.

Material used.—Ten diseased larvæ from sample No. 5863, macerated in 250 c. c. of a 50 per cent sugar sirup.

First appearance of disease noted.—May 31, 1918, three days after inoculation.

Age of larvæ first attacked.—Three to four days after hatching from the eggs.

Colony G (fig. 2), hybrids, soon succumbed to the infection, the first diseased larva appearing three days after infection, the gross diagnosis being confirmed by the finding of *Bacillus pluton* on microscopic examination. The spread of the disease was rapid, the disease being present in only one comb on the third day and in seven combs on the seventh day. All of this early spread took place in brood unsealed

at the time of infection. The first high peak of the disease coming on the nineteenth day was followed by a slight improvement, when for a time the house cleaning exceeded the occurrence of fresh disease. This was probably due to the stimulus of the increasing honey

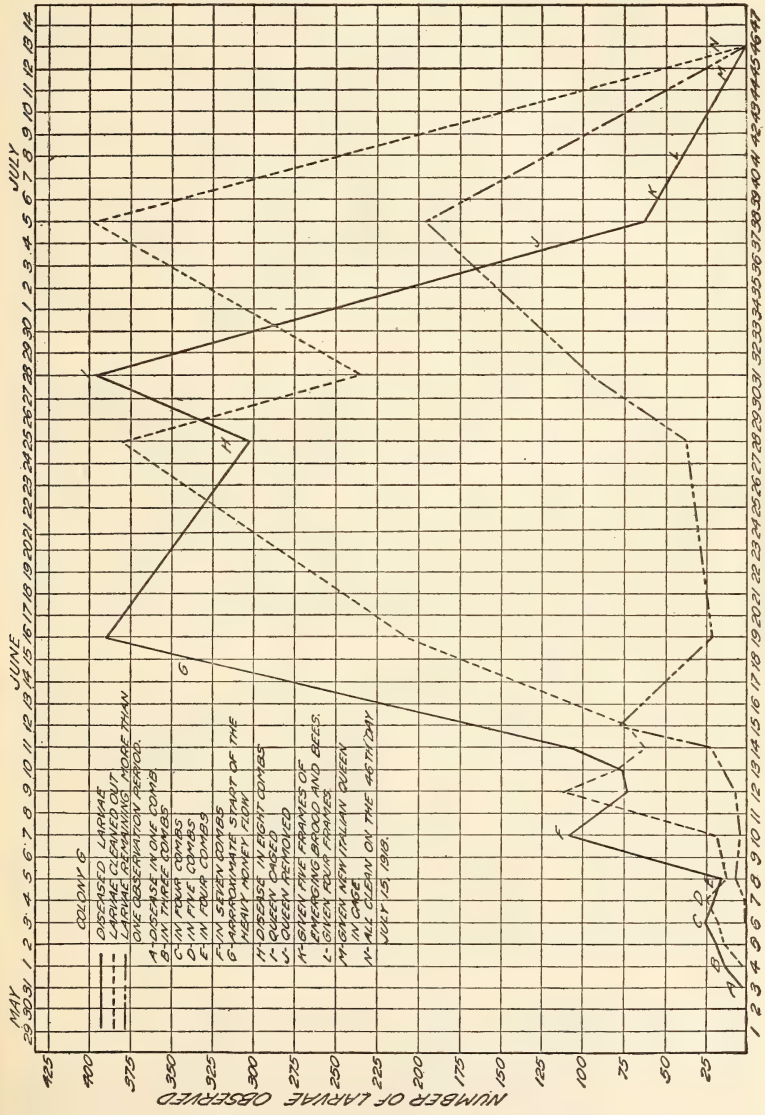

FIG. 2.—The course of European foulbrood in colony G.

flow. But as soon as the next series of eggs hatched, the disease again gained the upper hand, reaching another higher peak on the thirty-first day, at which time it was deemed necessary to start treatment. It had become evident that the colony was being overrun by the disease. More and more dead larvæ were being allowed to re-

main in the cells for several days without being cleaned out. Also more larvæ nearly ready for pupation were being affected. Most of these instead of remaining coiled were inclined to extend on the lower side wall in a brownish gray, slimy mass and exhibited a tendency to be viscid. At this stage of decomposition, when a stick is inserted the mass forms a coarse granular band for a short distance and then breaks so as to form droplike masses, but does not stretch out in a fine thread. These larval masses dried down to rubbery dark brown scales something like American foulbrood scales in appearance, but different in consistency. These scales could be removed quite easily and would bend like a piece of partially granular old rubber. They also lay irregularly placed in the cells, often spirally extended, while American foulbrood scales are uniformly on the lower side wall. The bacteriological explanation for this abnormal characteristic will be discussed later under bacteriological observations.

The predominance of these rubbery masses and scales increased as the disease progressed and the bees seemed to make little attempt to clean them out, even after the queen was caged on the thirty-first day, thus shutting off any increase of fresh larvæ, or even after the queen and all queen cells were removed on the thirty-seventh day. On the thirty-ninth and also on the forty-first day, five and four frames, respectively, of emerging brood and Italian bees were united with this colony, but it was not until a new Italian queen, confined in a cage, had been hung in on the forty-fifth day that a final complete cleaning up was made.

This new queen was not accepted, however, and a young queen was raised from the brood that was added to this colony, so that further observations were ended here although the virgin queen was killed and another Italian queen introduced. This colony was reported healthy, however, about the middle of August.

The hybrid bees seemed to lack ambition to fight the disease. When combs were removed from the colony, the bees never were observed to be working in the cells, and paid little attention to material partially drawn from the cells and crushed.

COLONY F

Race.—Italian, possibly with some slight hybrid blood.

Queen.—1917, fairly good condition.

Bees.—Workers, good color, fairly quiet, drones inclined to be darker.

Condition of colony at time of infection.—Brood in three frames, a little more than one-third sealed. Bees covering about six frames. Building up well. Proportion of field bees to nurse bees about equal.

Date of first infection.—May 31, 1918.

Material used.—Ten diseased larvæ from sample No. 5874, macerated in 250 c. c. of a 50 per cent sugar sirup.

First appearance of disease noted.—June 4, 1918, four days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

Colony F (fig. 3), which was the next one to be infected, although not as strong as colony G, was of Italian stock and did not show the appearance of disease until one day later. On the fourth day one cell appeared in each of two combs. It was not until the twenty-

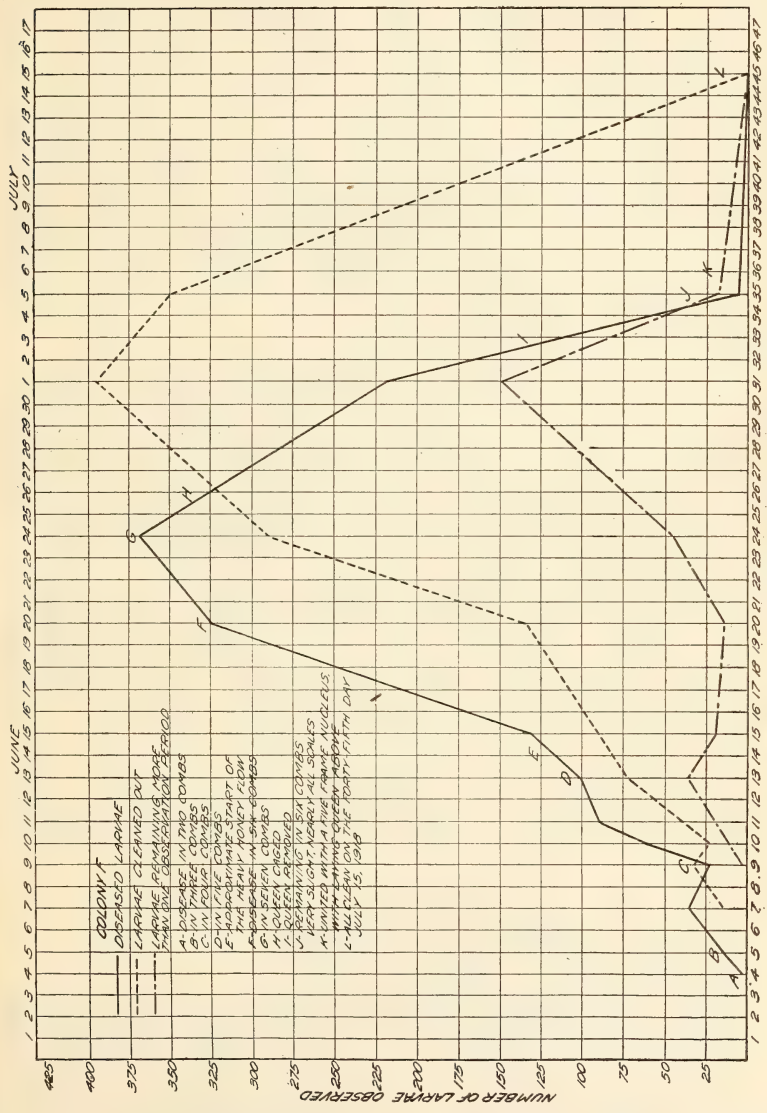

Fig. 3.—The course of European foulbrood in colony F.

fifth day that the disease had spread to seven combs, the total number of diseased larvæ being, as a whole, less than in the hybrid colony. There was not brood in all seven combs at the time of infection, but the brood increased faster than the disease spread. After the twenty-fourth day a permanent improvement began to be manifest.

This improvement continued after the queen was caged and became more marked after she was removed from the colony.

These bees were better house cleaners as well; the appearance of larvæ remaining over more than one observation period did not become evident until after the ninth day, compared with the sixth day in colony G. At no time were there as many of the larvæ nearly ready to pupate that were gummy or rubbery. Even though this colony was on the average weaker than colony G all the time, it handled the disease much better. It was 14 days before colony G had cleaned up to such an extent that it was deemed safe to introduce a new queen, while in colony F, with the Italian bees, the combs were so nearly cleaned of everything but a few old scales that a five-frame nucleus with a new Italian laying queen was united with this colony after a 10-day queenless period and in 9 days more everything was absolutely clean and the queen was laying in the combs that had had disease in them.

When an observation was made nine days after the new queen's eggs were first noted, it was found that there was a slight recurrence of disease in three of the combs. But, unfortunately, at the same time, queen cells and no eggs were found, denoting that for some reason this queen had not been accepted. Therefore the queen cells were all removed and a new queen was introduced. Although the author's observations ended of necessity soon thereafter, it was reported to him that this colony was doing nicely later in August and was perfectly healthy. If the first new queen had not disappeared, it is quite probable that as soon as a sufficient number of her bees had emerged they would have cleaned up the recurring disease in the same manner as was done in colony J, which will be mentioned later.

Several times in this colony, during the cleaning-up process, bees were watched in the act of sucking up juices of diseased larvæ that had been partially removed from the cells with the aid of forceps.

COLONY H

Race.—Hybrid, a division of Colony G, hybrid.

Queen.—1918. Of their own raising. Poor.

Bees.—Dark hybrids, almost black, excitable.

Condition of colony at time of infection.—Brood in three frames, a few eggs in one, only one small patch sealed, the remainder from eggs up to 4-day larvæ. Bees covering about five frames. Fairly good proportion of nurse bees.

Date of first infection.—July 1, 1918; second infection, July 8, 1918.

Material used.—20 old, dried, rubbery, diseased scales from sample No. 5898, macerated in 250 c. c. of a 50 per cent sugar sirup, colored with eosin.

First appearance of disease noted.—July 5, doubtful. Positive July 8, 7 days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

Colony H (fig. 4) was treated as a double experiment. The infection of this colony was not started until after the honey flow had come on quite heavily. Also, instead of freshly diseased larvæ, old brown rubbery scales were used that showed *Bacillus pluton* present microscopically, but were heavily overgrown by *Bacillus alvei*. It was desired to learn whether these scales were still infectious, so that nurse bees working on them, cleaning them out, might get infective material on their feet and mouth parts which could be carried to healthy larvæ. This was noted later in the observation hive, where, under the magnifying glass, bees were seen trying to remove some of these rubbery scales, first moistening them with their tongues and then pulling at them with the mandibles and front feet.

This colony, which was marked hybrid and weak, was slow in developing the disease, partly because of the diluting effect of the

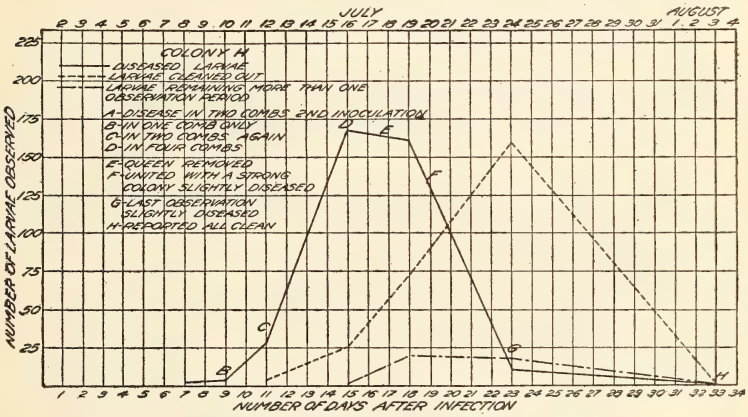

FIG. 4.—The course of European foulbrood in colony H.

heavy honey flow and probably partly because there was a smaller number of infectious organisms present in the scales than in fresh larvæ. This is explained by the fact that the secondary putrefactive invading organisms would tend to kill off the primary organism, because of the accumulation of the products of the putrefactive action.

On the seventh day before the disease was first noted, a second infection of scales macerated in sugar sirup was given this colony to counteract the effect of these retarding factors. However, later on the seventh day, diseased larvæ were found, and from then on the disease started to spread and increase irrespective of the heavy honey flow, exhibiting all the symptoms and tendencies shown in colony G, of which this colony was a division before infection.

On the seventeenth day it was necessary to remove the queen and start treatment, but what was taking place was evident. This removal of the queen did not seem to have a very marked effect on the house

cleaning until the colony was united with colony I, a slightly diseased Italian colony. They then began cleaning the H combs, and the combined colony was reported clean in August.

COLONY A

Race.—Italian with some possible slight hybrid blood.

Queen.—1918. Of their own raising.

Bees.—Workers, good color; fairly quiet. Drones, some slightly darker than pure Italians.

Condition of colony at time of infection.—Brood in seven frames about half sealed. Bees covering about nine frames with a good proportion of young nurse bees. Colony strong and building up.

Date of first infection.—July 2, 1918. Second infection, July 6, 1918.

Material used.—First, 20 diseased larvæ from sample No. 5937 macerated in 250 c. c. of a 50 per cent sirup, colored with eosin, abnormally heavy infection; second infection, 20 diseased larvæ from sample No. 5953 in 250 c. c. of uncolored sirup.

First appearance of disease noted.—July 8, 1918, in drone brood, six days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

Colony A (fig. 5) was a fairly strong colony of Italians. Like colony H, it was infected after the heavy honey flow had started and was

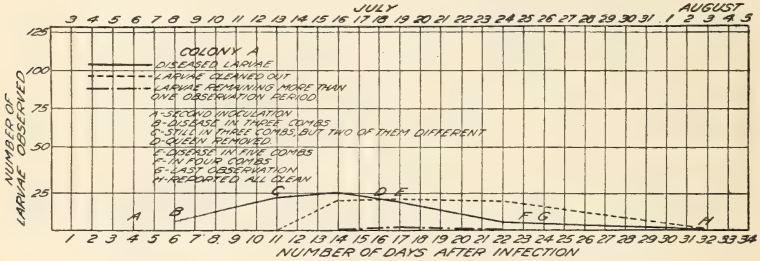

FIG. 5.—The course of European foulbrood in colony A.

given twice the amount of infective material colonies F and G received. Nothing having appeared on the fourth day, a second infection of the same amount was given. On the sixth day 6 diseased larvæ were seen in three combs. This colony, however, was so strong that the disease obtained very little foothold, and from the fourteenth day began to decline, or at least failed to make further gains. As a side experiment in this colony a comb of eggs laid by an Italian queen was placed in between two combs showing disease. If there is anything in the belief that Italian stock is more resistant to disease, the larvæ in this comb should not have developed the disease, or at least not so soon. However, on the sixth day one or two larvæ showed disease, increasing slightly in numbers for a few days until the observations were of necessity stopped. It was intended to perform this experiment with several variations, such as placing eggs laid by an Italian queen in a diseased hybrid colony and placing eggs from

a hybrid queen in a diseased Italian colony, but the presence of the heavy honey flow made it impracticable to carry the matter further.

This colony A cleaned up readily after removal of the queen and was reported all healthy in August. Although a new queen was given to them it is probable that a period of queenlessness and the returning of the same queen would have answered just as well.

COLONY I

Race.—Italian.

Queen.—1917, fairly good condition.

Condition of colony at time of infection.—Seven frames of emerging brood well covered with young bees. A strong 8-frame colony.

Date of first infection.—July 10, 1918.

Material used.—Thirty diseased larvæ from sample No. 5959, macerated in 250 c. c. of a 50 per cent sugar sirup. This was abnormally heavy infection of diseased material.

First appearance of disease noted.—July 15, 1918, five days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

This colony was infected during the heavy honey flow, but although given a heavy infection it had sufficient strength, aided by the heavy honey flow, to prevent the disease from spreading. On July 15 there were a few diseased larvæ in two combs. On July 24, 14 days after inoculation, there were only a few diseased larvæ in three combs. This was after the queen had been removed on July 18 and the colony had been united with colony H on the 20th.

An interesting observation was that under the magnifying glass the methods of the nurse bees in sucking the juices from dead diseased larvæ and the pulling of the skins out to carry them away could be noted. No bee worked very long at a time on one larva. One after another worked until all was completed.

SUMMARY OF PREVIOUS EXPERIMENTS

Table I gives a partial summary of the data thus far described.

TABLE I.—*Showing the first appearance of disease noted after infection. Also the number of combs showing infection and the spread of the infection from comb to comb in the various colonies under observation*

Colony.	Date infected.	Days after infection.																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
		Number of combs.																											
	1918																												
G[1]	May 28	..	..	1	3	..	4	5	4	..	7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	8
F[1]	May 31	..	..	..	2	3	..	..	..	4	..	..	..	5	..	..	..	..	..	..	6	..	..	..	7	..	..	..	..	..
H[2]	July 1 to July 8	..	..	..	..	..	..	2	..	1	..	2	..	..	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..
A[2]	July 2 to July 6	..	..	..	..	..	3	..	..	..	..	..	..	..	..	..	..	5	..	..	..	..	..	4	..	..	..	..	..	..
I[2]	July 10	..	..	..	..	2	..	..	..	..	..	..	..	..	3	(Different from first two.)														

[1] Experiments started before the beginning of the heavy honey flow.
[2] Experiments started after the beginning of the heavy honey flow.

In the first group, colonies G and F, it is quite apparent that the Italian bees, colony F, made the better showing, even though the hybrids were the stronger colony in the beginning. As may be seen from a comparison of the two plots in figures 2 and 3, in colony G wherever there was a lag in the house cleaning there was marked increase in the number of larvæ remaining over more than one observation period, and these increased until strengthening treatment was started. On the other hand, in colony F these were removed almost entirely by the time the strengthening treatment was started. The Italians did not allow the disease to appear as soon or to spread as rapidly, cleaned house better, left fewer larvæ to dry down to the brownish rubbery scales, and responded to the increased honey flow and treatment much more readily.

In the second group, colonies H, A, and I, the Italian colonies A and I again made the best showing. With the added diluting effect of the honey flow, they allowed the disease to gain no foothold whatever, while the hybrids, though aided by the honey flow, soon succumbed and allowed the disease to gain on them. It is evident that the Italian bees are much more vigorous house cleaners. In several instances, toward the end of the egg laying of the old queen, and well along in the progress of the disease, cells were noted on these diagrams which had previously contained diseased larvæ, but which had been cleaned out, and then in which disease had reappeared after other eggs had been laid and hatched in them. They were cells in which fresh nectar had not been placed between the two series of larvæ.

It was also noted that as the honey flow increased and as the brood became more scattered from the effects of the disease, more and more fresh nectar was placed in the brood nest in cells from which dead larvæ had been removed. Most of this nectar, however, was moved up later, particularly after the bees began preparing the brood nest for a new queen in the process of treatment. That the advent of a heavy honey flow was effective in controlling the disease is evident, particularly in the length of time between the infection and the first appearance of disease. The data, however, show little difference in the resistance to infection, or so-called immunity, being slightly in favor of the Italians, if there is any difference at all.

Disregarding the effect of the honey flow, the period of incubation of the disease is apparently between 3 and 4 days. However, it was noted that after *Bacillus pluton* was first observed it was anywhere from 24 to 48 hours before many characteristically diseased larvæ were observed. Therefore, the actual period of incubation is probably from 24 to 48 hours.

SUPPLEMENTARY OBSERVATIONS

STUDY OF NATURALLY INFECTED COLONIES

As a supplementary study to the preceding artificial infection experiments, some observations were made upon the behavior of naturally infected colonies undergoing treatment. Through the kindness of W. L. Bean, of McGraw, N. Y., it was possible to make a series of such observations. In his apiary of about 30 colonies, all hybrids, the majority were diseased when observed June 8, 1918. Soon thereafter Mr. Bean kindly loaned two of these diseased colonies to be carried to Ithaca for closer observation. Mr. Bean at once started treating his bees, requeening with Italian stock by the method of introducing a queen cell almost ready to emerge. Apparently, this method was successful, for in the latter part of July Mr. Bean reported all treated colonies healthy and some 800 pounds of surplus honey.

COLONY J

Race.—Hybrid.

Queen.—Queenless at time of arrival at Ithaca. Was poor hybrid of own raising, probably reared while disease was present in the colony.

Strength in spring.—Weak.

Strength at time of treatment.—Scattered brood in eight frames. Weak in bees, particularly in nurse bees.

Approximate date of disease first noted.—May 31, 1918.

Date of start of treatment observations.—June 16, 1918.

This colony made no effort to clean up, even though they had lost their queen shortly before being brought to Ithaca. On the 18th of June six frames of Italian bees and emerging brood were placed on top of it. At once house cleaning started, a reduction of 50 per cent being noted in the fresh, moist, melting larvæ within 24 hours. In this colony it was interesting to watch the bees doing the house cleaning, particularly when diseased larvæ in various stages of decomposition were partially withdrawn from the cell with a pair of forceps. With the aid of a powerful hand magnifying glass it was easy to watch them suck up the juices of the dead larvæ, even those which had decomposed to the extent of being a coffee brown in color and viscid in consistency. No bee would work long on a larva but would back off and wipe her tongue thoroughly with her front feet. It is conceivable that this might contaminate her, making possible carriage of the infection to the next larva fed, even though the juices of the diseased larva were not actually fed to the healthy one. The majority of bees engaged in this work were the Italians. From these and other observations of a similar nature there is no doubt that the contamination of the mouth parts is the primary method of spreading the disease inside the colony.

On June 25 an Italian queen was introduced in a cage with candy even though a few scales were still present. This was fully 10 days after the colony had lost its queen, if not a little longer. On June 27 the queen was out and laying in one comb. Eight days later, on July 5, a recurrence of disease was noted, one larva being discolored and sunken, showing *Bacillus pluton* on microscopic examination. From that time on, for about 20 days after the first eggs of this queen were noted, one or two new diseased larvæ appeared at each observation, the number decreasing, however, until about the twenty-sixth day when they had all disappeared. As the new young Italian bees increased, the disease decreased, until a point was reached where they were in the predominance and had eliminated the disease by their activity. This was also observed in colony F.

COLONY K

Race.—Hybrid.

Queen.—1917. Dark hybrid of their own raising, probably from diseased stock.

Strength in spring.—Weak.

Strength at time of treatment.—Eight frames of scattered brood and hardly enough bees to cover them.

Approximate date of disease first noted.—May 31, 1918.

Date of start of treatment observations.—June 20, 1918, at which time the queen was removed.

Colony K, when it was brought to Ithaca, was so weak that it would soon have died. The bees made no attempt to clean out larvæ that had been partially pulled out of the cells with forceps and crushed. On June 26, six days later, they were still showing freshly diseased, moist, melting larvæ from eggs laid by the old queen, just before removal. At this time five frames of emerging brood and Italian bees were given this colony. On the 27th a new Italian queen was hung in with the cage closed. The presence of the new queen, however, seemed to give added impetus to the house cleaning so that by July 1, 11 days after removal of the queen, they were practically cleaned up and the cage was opened with candy in the opening. Further observations on this colony were ended because they refused to accept this queen. By the time another queen finally was accepted and was laying on July 18, it was too late, as the season's work was closed by the 23d.

BEHAVIOR OF BEES IN CLEANING CONTAMINATED CELLS

On June 6, 1918, a sample was received for diagnosis (No. 5898), consisting of an entire brood comb, containing quite an area of capped honey. About one-half of each side of the comb contained a large number of dead and diseased European foulbrood larvæ, in stages varying from the yellowish, moist, melting larvæ to dried rubbery scales of which there was quite a large proportion. This was

the same sample from which infectious dried scales were used to infect colony H. After this comb had remained in the laboratory, wrapped in paper for about 3 weeks, it was placed in the strong colony in the observation hive. The frame was first placed in the middle of the hive for about an hour and was then removed to the outside, where the work of the bees on it could be watched. A large number of what appeared to be young nurse bees were already hard at work on the dried diseased material. The bees, working on the dried gummy masses, would wet the mass with their tongues for a while and then tear at them with their mandibles, at times removing pieces large enough to be seen from the outside. Often these small pieces were apparently dropped to the bottom board. No one bee worked long at one place. Those bees working particularly on the fresh, moist material, when leaving, would carefully wipe their tongues with their front feet, thereby transferring some of the infection to them. Other bees were at work carrying away the larger, more easily removable dead masses. The entrance also was watched to see if any of this material was carried out. Several bees were observed carrying out portions of dead larvæ or pupæ. One bee carried a piece about 2 yards before dropping it. Others dropped what they were carrying soon after leaving the entrance, but on examining the surface of the ground about the entrance, very little material could be distinguished, so that apparently most of the material removed must have been carried some little distance before being dropped. After about an hour's work it was apparent that considerable progress had been made. This comb was removed before it was entirely cleaned and later placed in another healthy colony for observation. It was quickly cleaned up and quite a bit of nectar placed in it and, eventually, several square inches of brood. Observations, however, had to be stopped before any appearance of recurrence was noted. This same observation hive was given one or two other diseased combs to clean, but with the repeated probable infection from these sources the colony was so strong that no disease was noted in it during the entire season of observations.

POSSIBLE INFECTION THROUGH QUEEN

Colony M was a small nucleus made to receive the old queen from diseased colony K from McGraw, N. Y. The queen was introduced on June 20, 1918. For a while she laid fairly well, it being necessary to add one or two more combs. But later her brood became more and more scattered. Finally, on July 8, there was observed one dead larva, which looked suspicious, but which, on microscopic examination, proved to be negative. On July 10, however, one definite cell appeared and several other slightly yellowish, abnormally colored larvæ. This dead larva contained *Bacillus pluton*. From then on until this queen was killed and the colony united with another dis-

eased colony, more discolored larvæ appeared, showing definitely the development of the disease. As far as could be seen the only source of infection was the queen which had come from a diseased colony.

This occurrence had been observed previously by the author while employed at the Massachusetts Agricultural Experiment Station. During the summer of 1916 eight queens taken from diseased European foulbrood colonies were introduced into isolated, healthy nucleus colonies. Of these eight nuclei three developed European foulbrood, two were doubtful, and three remained healthy. Several such instances have been mentioned in the literature of beekeeping.

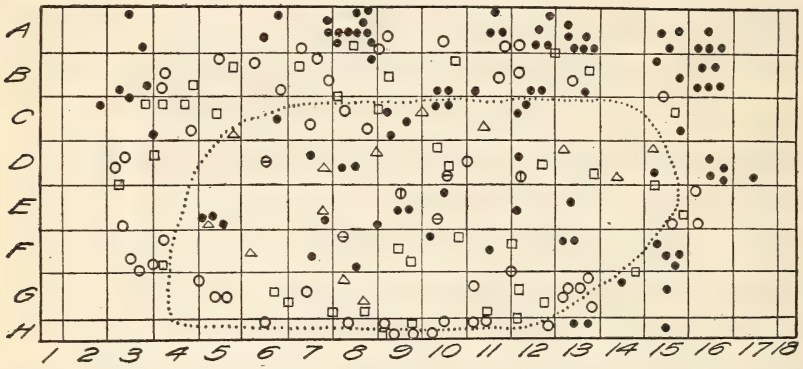

FIG. 6.—Distribution of cells containing infected sugar sirup and subsequent spread of the disease in a comb taken from colony H. . . . . Area covered by brood at time of infection, mostly unsealed. • Location of cells containing infected colored sugar sirup on July 3, 1918. ⦶ First positive diseased larvæ noted, 2 on July 8. ⊖ Number of new diseased larvæ (4) on July 10. △ Number of diseased larvæ (13) on July 12. □ Number of diseased larvæ (39) on July 16. ○ Number of diseased larvæ (52) on July 19.

DISTRIBUTION OF INTRODUCED INFECTED MATERIAL

An interesting experiment was carried out with sugar sirup, colored by a small amount of a harmless anilin dye, eosin, used as an indicator, which gave to the sirup a bright red color. The object of this experiment was to determine where the sirup, or, more important, where fresh nectar is first placed in the hive and combs. On May 27 two colonies were fed this colored sirup from above some time before the heavy honey flow from clover started. The results were striking, for in nearly every case the colored sirup was easily discernible in the cells and the greatest part of the sirup was located in quite a definite area. These colored cells were either scattered among the cells containing the larvæ or were placed in a ring of cells adjacent to the brood area toward the top of the comb, little being placed with the solid stores (fig. 6). Furthermore, for nearly 36 hours after the feeding practically all the young nurse bees showed a marked pinkish discoloration of the anterior end of their abdomens, denoting the dis-

tention of the honey stomachs by the retention of the colored sirup therein. About half the bees in the hives were discolored in this manner. After a day or so, however, this begin to disappear. Also the number of cells showing the pink discoloration began to disappear. Evidently the sirup had been moved up, worked over, and mixed with other nectar or consumed.

Later, some time after the heavy honey flow had started, shortly after July 1, two more colonies were fed colored sirup, this time infected with diseased larvæ macerated therein.

In these cases the discolored abdomens were noted about as before, but the colored cells were less numerous and the color less striking. The location of the colored cells was similar to that in the former experiment; that is, mainly in the brood area or just contiguous to it and mostly above. The outside combs, containing considerable honey, showed scarcely any of the colored cells. This time these colored cells disappeared sooner, showing that the infected material must have been much diluted quite soon after being taken up from the feeding dishes.

Figure 6 shows the method of plotting the location of diseased larvæ in the combs and also the location of the cells containing the colored sugar sirup. As will be noted, a fairly large proportion of these cells are located within the area of brood at the time of feeding. It is interesting to note the tendency of diseased brood to form concentric circles, showing the two series of larvæ occurring between the dates noted. The spreading was from two cells at first to quite a large number at the last observation shown.

AGE AT WHICH LARVÆ ARE INFECTED

In previous observations it was constantly noted that the larvæ affected by European foulbrood were regularly at least 4 days old, the age at which the coiled larvæ completely fill the bottom of the cells. Occasionally a slightly younger and smaller larva would become diseased, but this was not the common occurrence. Furthermore, in the cases where the colored sirup was fed the bees, within 24 to 36 hours quite a number of larvæ averaging 4 days old could be seen discolored from having been fed this sirup, while it was noticeable that the younger larvæ under 3 days old never showed the discoloration. These colored larvæ were examined in a smear under a microscope, but the infecting organisms, being comparatively few in number, had not increased sufficiently at that time to be apparent.

The question now arises as to the age at which the larvæ first are fed nectar or infected material. There has been much controversy over the subject of composition and source of the larval food, but as yet no conclusive scientific evidence has been presented. Irrespective of the question whether the food at various stages originates from glands or is regurgitated, it is apparent from these observations that

there must be a difference between the food which larvæ younger than approximately 3 days old receive and that fed to older ones. Otherwise the younger larvæ would also show the pink coloration. Von Planta (9) by chemical analyses, of questionable exactness, however, makes a division in the feeding of the larvæ at the age of 4 days, at which time the high protein and low sugar content change to lower protein and higher sugar content. These analyses would tend to coincide with the above observations, only it is probable that the change begins earlier.

Additional data upon this subject are recorded in Tables II and III, although the observations were primarily for another purpose. In order to obtain further information relating to a possible difference in resistance to disease between Italian and hybrid bees, a careful record was made of the time when eggs were first noted in empty combs after the infection of the colony and when larvæ first showed disease thereafter. In the case of comb Special No. 2, the eggs were laid by an Italian queen in a healthy colony and then placed in a diseased colony. Colonies F, A, and I were of Italian stock while colonies G, H, and J were hybrid. In the recurrence of disease all were given new Italian queens. As has been mentioned before, as soon as the bees of the new Italian queens emerged in sufficient numbers the disease disappeared.

TABLE II

THE FIRST APPEARANCE OF DISEASE IN COMBS IN WHICH EGGS WERE LAID AFTER THE COLONY WAS INFECTED

Colony and comb No.	Number of days after eggs were first noted in comb.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
G 4										×				
G 6							×							
G 7						×								
G 8							×							
F 3							×							
F 4								×						
F 7						×								
H 3									×					
H 5									×					
A 2											×			
A 4											×			
A 5						×								
A 7								×						
A 8											×			
Spec. 2						×								
I 3									×					

RECURRENCE OF DISEASE AFTER EGGS OF A NEW QUEEN WERE FIRST NOTED IN THE COMBS

J 2														×
J 3														×
J 4							×							
J 5								×						
J 6a							×							
J 6b											×			
J 7										×				
J 8							×							
F 3									×					
F 4									×					
F 7									×					

TABLE III.—*Average time, under various conditions, in which disease becomes apparent in a colony after infection with European foulbrood. (Averages taken from Table II)*

	Before the heavy honey flow.
	Days.
Colony G, hybrid	7½
Colony F, Italian	7
Average of these two	7¼
	During the heavy honey flow.
	Days.
Colony H, hybrid	9
Colony A, Italian	8⅝
Colony I, Italian	9
Average of these three	8 11/12
	Recurrence of disease, after treatment, during honey flow.
	Days.
Colony J, hybrid originally	9¾
Colony F, Italian originally	9
Average of these two	9⅜

The data shown, particularly in Table III, tend to disprove the theory that Italian bees have a natural immunity or resistance. If a larger number of observations could have been made, the variation would have appeared less. The effect of the honey flow is evident, however.

When it is a question of the age at which the larvæ are fed material that contains infection, these figures are significant. In the life history of the bee, 3 days are spent in the egg and from 5 to 6 days as larva before capping, making a period of 9 days in all. After 3 days in the egg and after having been fed predigested food for 3 days, with the additional 24 to 48 hour period of incubation, as was observed earlier in this paper, the larva ought to show disease from the fourth to the fifth day after hatching, or the seventh to eighth day of its existence, if Von Planta's assumption is correct. From actual observation this was found to be true and from observation of the averages in Table III it is seen that the first appearance of disease occurs between the seventh and ninth days, varying with the conditions of the honey flow.

Referring to Dr. Miller's theories, it is hard to believe that there is not plenty of highly infectious material left in the colony after a 5 or 6 day period of queenlessness. Aside from actual observations of moist, yellow, melting larvæ present more than 6 days after the

queen has been removed, the juices of which the workers sucked up with avidity, the final eggs laid will be just at the stage where the disease first appears; that is, 3 to 4 days after hatching, at the end of a 6-day period. Furthermore, even though the nurse bees do not feed to healthy larvæ the material that is taken up in cleaning out the cells in varying stages of decomposition, infection, even from scales, may be carried on the feet, mouth parts, and tongue, particularly, as was definitely shown with colony H, since these scales are infectious. The period of queenlessness and the consequent house cleaning are absolutely dependent on the strength of the colony. A strong colony cleans up rapidly, particularly after the introduction of the new queen in a cage plugged with candy. A weak colony, on the other hand, has not sufficient bees to clean even after complete introduction of a queen, and the disease soon appears again. Under average conditions, therefore, it would appear unsafe to allow less than a 10-day period of queenlessness in treatment of European foulbrood.

MICROSCOPICAL BACTERIOLOGICAL OBSERVATIONS

A large number of microscopic examinations were made of larvæ under various conditions for the positive presence of the characteristic groups of *Bacillus pluton*. These examinations were made mainly as a check on the gross observations of the first appearance of the disease. Cover glass smears were made of crushed larvæ, stained with carbol fuchsin and mounted in Canada balsam. These examinations were made at regular intervals after the colonies were infected, larvæ of all ages being examined.

It was found in the smears of those larvæ showing the first slightly abnormal symptoms that *Bacillus pluton* was the only organism present. This substantiates White's (12) observations that before the disease could be detected by gross examination, by a histological study of sections of larvæ during the period of incubation it was demonstrated that "in the production of the disease *Bacillus pluton* was the first invader of the healthy larvæ."

As the disease advanced in the various colonies, observations were made of larvæ in various stages of decomposition. The bacterial content was found to vary with the change of appearance of the larvæ during decomposition. The presence of these secondary invaders easily explains the atypical appearance of certain types of European foulbrood that heretofore have been very confusing to the beekeeper.

For a short time after the death of the larva, the color remains a moist, creamy-grayish yellow. This is during the period when *Bacillus pluton* and such occasional secondary invaders as *Streptococcus apis* or *Bacterium eurydice* and other organisms, which do not form

spores, are predominant as described by White (12) and McCray (4). Soon the putrefactive spore-forming organisms increase in number, *Bacillus alvei*[1] being the one most commonly found. This is seen particularly in the case of the more mature larvæ, which when dying extend more or less irregularly in the cells, becoming the grayish brown slimy masses which develop into the dark brown granular rubbery scales. This fact has been observed for a long time in the many samples which have been received for diagnosis. A partial description of these scales and of the presence of *Bacillus alvei* in them is given by McCray and White (5), but the experimental observations described in this paper added to diagnostic observations show that this condition is generally much more pronounced and common than described by these writers from laboratory observations. The rapid increase and peculiar process of decomposition of *Bacillus alvei*, after the death of the larva, often to the exclusion of all other organisms, accounts for this abnormal appearance. In the case of American foulbrood, almost never is any other organism found associated with the disease but *Bacillus larvae*, the cause of the disease. This accounts for the constancy of the symptoms as compared with the variation of symptoms in European foulbrood where there may be several secondary invaders.

Furthermore, in making the smears of the diseased larvæ upon cover glasses, the peculiar whitish saclike extrusion of the larval intestines was often noticed on crushing the larvæ preparatory to smearing, which White (10) describes as a gross diagnostic character. When this sac was removed and smeared separately, it was always found to be heavily loaded with *Bacillus pluton*. Therefore it is safe to assume that the intestinal tract is the primary focus of infection, while the secondary putrefaction takes place mostly in the body tissues of the dead larva.

Coincident with the microscopic examination of larvæ, several examinations were made of the contents of the ventriculus, rectum, and in a few cases of the honey stomach and mouth parts of bees. These bees were presumably nurse bees taken from diseased combs, some in the very act of sucking up the juices of dead diseased larvæ. Although insufficient observations were made to give conclusive evidence, some interesting information was obtained.

As may be seen from Table IV, the number of cases where *Bacillus pluton* or other organisms associated with infectious material were found in the intestinal contents is not very large. However, of more

[1] *Bacillus alvei* originally was supposed to be the primary cause of European foulbrood, but has been proved by White and others to be only a common secondary invader. *Bacillus alvei* has purely putrefactive functions. From its cultural and biochemical characteristics, *Bacillus alvei* apparently belongs to the common *Bacillus subtilis* (hay bacillus) group of spore-forming organisms, all having mainly putrefactive functions.

importance probably, *Bacillus pluton* was found in a smear made from the mouth parts of a nurse bee and also in the contents of the honey stomach of another. If these observations had been carried out systematically, instead of only casually, it is expected that much more positive data might have been obtained along these lines, owing to what is known already of the habits of house-cleaning bees working on diseased material.

TABLE IV.—*The results of the microscopic bacterial examination of the contents of the intestinal tracts of nurse bees taken from diseased colonies*

Microscopic findings.	G.	F.	H.	A.	I.	J.	K.	Total.
Positive *Bacillus pluton*	1	2	2	.....	.....	.....	4	9
Negative *Bacillus pluton*	11	24	18	17	12	6	9	97
Bacillus alvei or doubtful *Bacillus pluton*	.....	3	1	.....	.....	7	.....	11

SUMMARY AND CONCLUSIONS

In arriving at the following conclusions an effort has been made to state them in a manner which will indicate the substantiation of previous observations made both in the laboratory and in the apiary. It may be noted that many of these conclusions are similar to some of the statements made in Farmers' Bulletin 975 in the summary of facts which apiary practice has brought out.

1. European foulbrood is an infectious disease. *Bacillus pluton* was found to be the primary invader, appearing in the intestinal tract of larvæ before death, contemporary with the first slightly apparent symptoms.

2. The variation in the appearance of the diseased larvæ after death is due to the presence or absence of secondary invaders.

3. The period of incubation for European foulbrood was found to be from 36 to 48 hours, although the gross symptoms usually do not become apparent in less than 3 or 4 days, varying with conditions of honey flow and strength of colony.

4. It has been noted in apiary practice that the first brood of the year usually escapes with little loss. During the first 5 to 7 days the spread of the disease in the colony after infection is slow, after which the increase is rapid under favorable conditions. The critical time, therefore, to detect the disease and start treatment is early in its course, thus making conditions unfavorable.

5. The evidence tends to confirm the theory that one of the ways the disease is spread in the colony is by the house-cleaning bees, and from colony to colony by their drifting. It is quite probable that the infective organisms are carried on the mouth parts and pedal appendages. The question of infection from intestinal contents or from

the source of larval food at various stages needs further substantiation.

6. Irrespective of strength of colony, the Italian bees were found to resist infection much better than hybrids and showed more ability to overcome the disease.

7. This apparent resistance of the Italian bees was observed to be largely due to the more vigorous house-cleaning characteristics rather than to a natural resistance or immunity to the disease. There was very little difference in the apparent period of incubation between the Italian and hybrid colonies, possibly a slight difference in favor of the Italians. Furthermore, it was noted that often there may be a slight recurrence of disease in the brood of the new Italian queen until a sufficient number of her bees have emerged to eliminate the infection by house cleaning. Apparently, infection is not always entirely removed by a period of queenlessness.

8. As a rule, requeening is necessary in the treatment of European foulbrood, except possibly in the strongest Italian colonies, which show only slight infection. Where a considerable quantity of disease is present, sufficient to require treatment, it was found unsafe to use a period of less than 10 days' queenlessness, due to the infectious condition of the diseased material remaining and the accompanying behavior of the colony.

9. The stronger the colony in Italian bees, the more rapid was the recovery.

10. A heavy honey flow tends to prevent infection from gaining a foothold. It also tends to eliminate the disease if present before the start of the heavy honey flow. This was found to be due to the effect of dilution on the infection because of the influx and direct feeding of the fresh nectar to the larvæ.

11. European foulbrood is a disease of weak colonies. It was found to be difficult effectually to infect any but the very weak colonies during the heavy honey flow. Therefore, colonies kept strong up to the time of the honey flow run very little danger of contracting European foulbrood. This and others of the facts observed are in exact harmony with facts already observed in apiary practice.

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[1] "Black brood" is an old name for European foulbrood.

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